The Role of MicroRNA in Inflammatory Bowel Disease

Sushila R. Dalal, MD, and John H. Kwon, MD, PhD

Dr. Dalal is a GI Fellow and Dr. Kwon is an Assistant Professor of Medicine in the Section of Gastroenterology of the Department of Medicine at the University of Chicago in Chicago, Illinois.

Address correspondence to:
Dr. John H. Kwon
Section of Gastroenterology
University of Chicago
900 E. 57th Street
KCBD 9118, Mailbox 9
Chicago, IL 60637;
Tel: 773-702-5958;
Fax: 773-702-2281;
E-mail: jkwon@medicine.bsd.uchicago.edu

Abstract: Inflammatory bowel disease (IBD) is the consequence of an abnormal immune response to environmental factors in genetically susceptible hosts. microRNAs (miRNAs) are small, 22-nucleotide, noncoding, single-stranded RNA molecules involved in the post-transcriptional regulation of 30% of protein-coding genes. Differential expression of miRNAs is described in multiple autoimmune-related conditions such as psoriasis, rheumatoid arthritis, lupus, and asthma. Recently, unique miRNA expression profiles have been described in epithelial cells of patients with active ulcerative colitis, Crohn's ileitis, and Crohn's colitis, as well as in the peripheral blood of patients with active ulcerative colitis and Crohn's disease. miRNA expression profiles also change in the progression from normal colonic tissue to dysplastic tissue, with unaffected tissue from IBD patients and inflamed tissue from IBD patients showing intermediate profiles. Understanding the role of miRNAs in IBD may lead to future insights into disease pathogenesis, diagnosis, and treatment.

Keywords
microRNA, Crohn's disease, ulcerative colitis, inflammatory bowel disease, gene expression

Crohn's disease (CD) and ulcerative colitis (UC) are the 2 predominant types of idiopathic inflammatory bowel disease (IBD). Both are often distinguished on the basis of history, physical findings, radiology, serum markers, and endoscopic and histologic extent and appearance of disease.[1] Up to 10% of IBD patients with colonic inflammation cannot be clearly classified into either group and are given the diagnosis of indeterminate colitis.[1] While both CD and UC are thought to arise in genetically susceptible individuals as a consequence of a dysregulated immune response to the environment, these diseases involve different pathophysiologic mechanisms, as evidenced by distinct genetic risk factor patterns and gene and protein expression signatures.[2-7] For example, CD and UC demonstrate distinct immune profiles, and they differ in terms of their associated circulating and intestinal immune cell types and Th1, Th2, and Th17 cytokine profiles.[8] CD is associated with increased production of the Th17-produced cytokine, interleukin (IL)-17, and the Th1-produced cytokines, interferon (IFN)-γ and tumor necrosis factor (TNF)-α.[9,10] In UC, an atypical Th2 response mediated by nonclassical innate natural killer cells with elevated levels of IL-13 has been observed.[11]

Several genome-wide expression studies indicate that the genes involved in immune responses are not the only genes that are differentially expressed in CD and UC.[4-6] In one study of 36 expression profiles of colonoscopic pinch biopsies, CD patients had 47 genes with increased expression and 30 genes with decreased expression, while UC patients showed increased expression of 51 genes and decreased expression of 81 genes.[6] While the gene expression patterns noted for CD and UC patients did show some overlap, distinct gene expression patterns were observed for multiple processes, including fibrosis, metabolism, biosynthesis, electrolyte transport, cell proliferation, and permeability.[4-6]

Investigations of IBD have been limited by an incomplete understanding of the mechanisms regulating gene expression and the complex interplay of factors that contribute to the etiopathogenesis of CD and UC. The importance of post-transcriptional regulation of gene expression in unique and overlapping disease phenotypes is becoming increasingly clear. One key post-transcriptional mechanism is the negative regulation of messenger RNA (mRNA) stability and protein translation via microRNAs (miRNAs), small (~22–24-nucleotide), noncoding, single-stranded RNA molecules.[12] This review focuses on the expression of miRNAs in the immune system of individuals with autoimmune diseases and their potential role in the pathogenesis, diagnosis, and treatment of IBD.

A General Overview of MicroRNAs

The first miRNA was described in 1993, when Lee and colleagues found that a gene locus known to control *Caenorhabditis elegans* development, *lin-4*, produces a 22-nucleotide, noncoding strand of RNA.[13] The lin-4 small RNA (later renamed miRNA) was found to have partial complementarity to the 3'untranslated region (UTR) of the lin-14 mRNA, and expression of the lin-4 small RNA decreased lin-14 protein expression.[14] Subsequent discovery of the let-7 miRNA in *C. elegans* and its wide conservation across many species, including humans, clarified the evolutionary significance of miRNAs.[15,16] Researchers then began searching for more miRNAs in flies, worms, humans, and other mammals. Since the observation of the first miRNA in humans, over 1,000 human miRNAs have been described.[17]

miRNAs are transcribed from intronic or intergenic regions as pri-miRNA transcripts.[18-21] The pri-miRNA transcript is processed by the double stranded-RNA–specific endonuclease, Drosha, in a complex with DGR8, into a ~70-base pair stem loop precursor miRNA (pre-miRNA).[22] The pre-miRNA is exported to the cytoplasm via exportin 5.[23,24] Once in the cytoplasm, the endonuclease Dicer cleaves the pre-miRNA into a small, double-stranded RNA duplex, and then the mature strand of the miRNA duplex is incorporated into the RNA-inducing silencing complex.[25-29] The miRNA can then bind to complementary sequences in the 3'UTR of target mRNAs, resulting in either degradation of the target mRNA or translational inhibition.[30]

Each miRNA can target hundreds of mRNAs within a given cell type, and a single mRNA is often the target of multiple miRNAs.[31,32] Thus, miRNAs contribute to the regulation of over 30% of protein-coding genes,[33] and miRNAs are involved in cell development, metabolism, cell cycle control, and fibrosis.[34,35] miRNAs have been extensively studied in multiple types of cancer and have been found to be regulators of tumor suppressors and oncogenes.[36] Studies of miRNAs in lymphoma and other hematologic malignancies have led to insights into the role of miRNAs in the immune system.[37,38]

MicroRNAs in the Innate and Adaptive Immune System

The role of miRNAs in both the innate and adaptive immune systems has been increasingly recognized.[39] The innate immune system is activated through recognition of pathogen-associated molecular patterns (PAMPs) by toll-like receptors (TLRs).[40] After recognizing a PAMP, TLRs bind adaptor proteins, such as members of the myeloid differentiation factor (MyD88) family, which leads to downstream activation of signal transduction pathways such as nuclear factor kappa B (NF-κB) and mitogen-activated protein (MAP) kinases.[41] These signal transduction pathways then lead to the transcriptional or post-transcriptional regulation of cytokines such as IFN-γ, IFN-β, or TNF-α.[41,42] In murine macrophages, the TLR ligands polyinosinic:polycytidylic acid (poly[I:C])and lipopolysaccharide (LPS) and the cytokine IFN-β induce miR-155 expression via the MyD88 or transiently induced factor (TRIF) adapter proteins and the Jun N-terminal kinase and NF-κB signaling pathways.[43] IFN-β induction of miR-155 requires TNF-α autocrine signaling.[43]

Once induced, miR-155 is involved in antigen presentation as well as activation of the LPS/TNF-α pathway.[44-46] miR-155 targets suppressor of cytokine signaling (SOCS)-1, a negative regulator of dendritic cell antigen-presenting capacity.[45] Loss of miR-155 in dendritic cells prevents T-cell activation via impaired antigen presentation and costimulation activity.[44] miR-155 also targets Fas-associated death domain protein, IκB kinase ε, and receptor (TNF receptor superfamily) interacting serine-threonine kinase 1, all of which are involved in the activation of the LPS/TNF-α pathway.[46] While miR-155 may decrease LPS/TNF-α signaling through these pathways,

this miRNA also acts directly or indirectly to increase TNF-α translation.[46]

miR-146 is also involved in TLR signaling in the innate immune system. In human monocytes, expression of miR-146a and miR-146b is induced by exposure to TLR ligands, LPS, peptidoglycan, and flagellin.[47] miR-146a reduces expression of 2 components of the TLR signaling cascade: TNF-receptor–associated factor (TRAF)-6 and IL-1 receptor–associated kinase (IRAK)-1. Thus, increased miR-146a expression leads to negative feedback that attenuates the TLR response. While miR-155 and miR-146 expression is increased in macrophages in response to LPS stimulation, miR-125b expression is decreased.[46] miR-125b targets TNF-α, and thus decreased expression of miR-125b leads to an increased inflammatory response due to elevated expression of TNF-α.[46]

miRNAs are also important in T- and B-cell development.[48] miR-150 regulates the transition from the pro– to the pre–B-cell stage, and overexpression of this miRNA prevents B-cell development.[49] The expression of most miRNAs in B cells is differentiation stage–specific, and changes in miRNA expression can be used to classify B-cell subpopulations.[37] miRNA expression was found to be highest in the nonreplicating naïve T cells and the relatively quiescent memory T cells, but it was significantly downregulated in actively dividing effector T cells.[50]

MicroRNAs in Immune-Mediated Disorders: Tissue Specificity and Common Immune Mechanisms

The growing understanding of the importance of miRNAs in innate and adaptive immunity has been accompanied by numerous studies identifying the differential expression of miRNAs in immune-mediated disorders such as psoriasis, atopic eczema, rheumatoid arthritis (RA), asthma, and systemic lupus erythematosus (SLE; Table 1).[51-56] An miRNA microarray analysis comparing the skin of psoriasis patients with that of atopic eczema patients and healthy controls revealed increased expression of 12 miRNAs and decreased expression of 17 miRNAs in psoriasis patients.[51] Increased levels of miR-146a in psoriatic skin leukocytes may affect TNF-α signaling via TRAF-6 and IRAK-1, as previously described in macrophages.[47,51] Modulation of TNF-α signaling via TNF-α inhibitors is an effective treatment for psoriasis, as this chemokine is involved in cross-talk between immune cells and keratinocytes.[51,57] In addition to differential miRNA expression in immune cells, keratinocytes in psoriasis patients also have unique miRNA profiles, one feature of which is increased expression of miR-203.[51] SOCS-3, a putative target of miR-203, is decreased in psoriatic skin,[51] and SOCS-3 deficiency leads to sustained activation of signal transduction and transcription (STAT)3 in response to IL-6, a cytokine present in psoriasis lesions.[51,57,58] Thus, suppression of SOCS-3 by miR-203 may lead to sustained STAT3 activation, infiltration of the skin with leukocytes, and the development of psoriatic plaques.[51]

Common miRNA profiles appear in RA as well, with increased expression of miR-155 and miR-146 in the synovial fibroblasts of RA patients compared to osteoarthritis patients.[52] Furthermore, miR-155 is increased in $CD14^+$ monocytes in the synovial tissues of RA patients compared to $CD14^+$ cells in the peripheral blood.[52] As in macrophages, TNF-α, LPS, and poly(I:C) treatments also increase miR-155 expression in synovial fibroblasts.[52] Increased miR-155 expression correlates with decreased expression of matrix metalloproteinase (MMP)-1 and MMP-3 in these cells.[52] Although direct targeting of miR-155 against MMP-1 and MMP-3 has not been established, this miRNA may have a regulatory role in controlling tissue damage induced by MMPs in RA.[52] miR-146a levels were also increased in $CD68^+$ macrophages, several $CD3^+$ T-cell subsets, and $CD79a^+$ B cells in RA synovial tissue.[53]

In SLE patients, miR-146a expression is decreased in peripheral blood leukocytes compared to healthy controls.[59] This decrease in miR-146a was associated with increased clinical disease activity and overactivation of the type I IFN pathway.[59] Comparison of the miRNA profiles in SLE patients, idiopathic thrombocytopenic purpura (ITP) patients, and healthy controls identified a group of 13 miRNAs that had the same expression pattern in SLE and ITP.[55] Three miRNAs (miR-184, miR-198, and miR-21) had differential expression in SLE compared to controls but were unaltered in ITP.[55] Six miRNAs were decreased in ITP but not SLE.[55] In kidney biopsies taken from lupus nephritis patients, 66 miRNAs were differentially expressed compared to controls.[56] These miRNAs were not the same ones identified in the peripheral blood mononuclear cells (PBMCs) of lupus patients.[55,56,59]

In asthma, *HLA-G* has been identified as an asthma susceptibility gene.[60] Binding of miR-148a, miR-148b, and miR-152 to the 3'UTR of HLA-G mRNA was confirmed with luciferase reporter assays.[54] Furthermore, a single nucleotide polymorphism (SNP) in the HLA-G 3'UTR influences the targeting of these miRNAs to this gene, which may account for the effect of the *HLA-G* genotype on asthma risk.[54]

Thus, unique miRNA expression profiles have been identified in several immune-mediated disorders. miRNA profiles of immune cells show differential expression of some common miRNAs (miR-155 and miR-146a) across different diseases, while tissue-specific expression appears

Table 1. MicroRNA (miRNA) Expression in Immune-related Diseases

Disease	Cell/tissue	Associated miRNAs	Reference
Psoriasis	Human skin	**Increased expression:** miR-146b, miR-20a, miR-140a, miR-31, miR-200a, miR-17-5p, miR-30a-5p, miR-141, miR-21, miR-142-3p, miR-106a, miR-203 **Decreased expression:** let-7e, miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524, miR-30c, miR-365, miR-133b, miR-10a, miR-133a, miR-22, miR-326, miR-215	Sonkoly E, Wei T, Janson PC, et al[51]
Atopic eczema	Human skin	**Increased expression:** let-7i, miR-199a, miR-29a, miR-27a, miR-146a, miR-21, miR-222, miR-20a, miR-24, miR-17-5p, miR-193a, miR-106b **Decreased expression:** miR-122a, miR-133a, miR-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-515-5p	Sonkoly E, Wei T, Janson PC, et al[51]
RA	RA-derived synovial fibroblasts	miR-155 induced by TNF-α, IL-1β, poly(I:C), LPS miR-146a induced by IL-1β and LPS	Stanczyk J, Pedrioli DM, Brentano F, et al[52] Nakasa T, Miyaki S, Okubo A, et al[53]
Asthma	Human bronchial epithelial cells and choriocarcinoma cells	miR-148a, miR-148b, miR-152 All target the 3'UTR of the *HLA-G* gene	Tan Z, Randall G, Fan J, et al[54]
Lupus nephritis	Human kidney tissue	**Increased expression:** miR-518c*, miR-23a, miR-638, miR-198, miR-583, miR-200c, miR-612, miR-516-5p, miR-142-5p, miR-320, miR-657, miR-184, miR-197_MM2, let-7e, miR-134, miR-494, miR-513, miR-575, let-7a_MM1, miR-658, miR-600, let-7a, miR-433, miR-185, miR-324-5p, miR-325_MM2, miR-662, miR-208, miR-130b, miR-30a-5p, miR-601, miR-622, miR-608, miR-195, miR-124a, miR-15b_MM1 **Decreased expression:** miR-296, miR-150_MM1, miR-365, miR-324-3p, miR-518b, miR-346, miR-637, miR-133a_MM1, miR-557, miR-615, miR-345_MM1, miR-642, miR-654, miR-484, miR-99a, miR-223, miR-611, miR-30d, miR-500, miR-663, miR-423, miR-381_MM1, miR-602, miR-210, miR-596, miR-486, miR-769-3p, miR-629, miR-92b_MM2, miR-150	Dai Y, Sui W, Lan H, Yan Q, Huang H, Huang Y[56]
Systemic lupus erythematosus	Peripheral blood mononuclear cells	**Increased expression:** HMP predicted miR-189, HMP predicted miR-61, HMP predicted miR-78, miR-21, miR-142-3p, miR-342, miR-299-3p, miR-198, mmu-miR-298 **Decreased expression:** miR-196a, miR-17-5p, miR-409-3p, HMP predicted miR-141, miR-383, HMP predicted miR-112, miR-184	Dai Y, Huang YS, Tang M, et al[55]

HMP=human predicted sequence; IL=interleukin; LPS=lipopolysaccharide; poly(I:C)=polyinosinic:polycytidylic acid; RA=rheumatoid arthritis; TNF=tumor necrosis factor; UTR=untranslated region.

Table 2. MicroRNA (miRNA) Expression in Ulcerative Colitis (UC) and Crohn's Disease (CD)

Disease	Tissue	Associated miRNAs	Reference
Active UC (compared to healthy controls)	Sigmoid colon biopsies	**Increased expression:** miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126, miR-195, let-7f **Decreased expression:** miR-192, miR-375, miR-422b	Wu F, Zikusoka M, Trindade A, et al[61]
Chronically active CD (compared to healthy controls)	Sigmoid colon biopsies	**Increased expression:** miR-23b, miR-106a, miR-191 **Decreased expression:** miR-19b and miR-629	Wu F, Zhang S, Dassopoulos T, et al[62]
Chronically active CD (compared to healthy controls)	Terminal ileal biopsies	**Increased expression:** miR-16, miR-21, miR-223, miR-594	Wu F, Zhang S, Dassopoulos T, et al[62]
Active CD (compared to healthy controls)	Peripheral blood	**Increased expression:** miR-199a-5p, miR-362-3p, miR-340*, miR-532-3p, miRplus-E1271 **Decreased expression:** miR-149*, miRplus-F1065	Wu F, Guo NJ, Tian H, et al[66]
Active UC (compared to healthy controls)	Peripheral blood	**Increased expression:** miR-28-5p, miR-151-5p, miR-199a-5p, miR-340*, miRplus-E1271, miR-103-2*, miR-362-3p, miR-532-3p, miR-3180-3p, miRplus-E1035, miRplus-F1159 **Decreased expression:** miR-505*	Wu F, Guo NJ, Tian H, et al[66]

to be unique to each disease state. Targeting and function of these tissue-specific miRNAs remains a topic of future investigation.

MicroRNAs in Ulcerative Colitis and Crohn's Disease

Unique MicroRNA Tissue Profiles

As in the target tissues of psoriasis and SLE patients, UC and CD patients also have unique miRNA expression profiles in their target organs (Table 2). While some differentially expressed miRNAs are common to other immune-related disorders, most are unique.

In a study conducted by our laboratory, sigmoid colon biopsy miRNA microarray profiles for healthy control subjects and patients with active UC, inactive UC, chronic active CD, irritable bowel syndrome, and microscopic colitis were compared. This comparison revealed that 3 miRNAs (miR-192, miR-375, and miR-422b) were significantly decreased in the UC tissues, while 8 miRNAs (miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126, miR-195, and let-7f) were significantly increased in active UC tissues.[61] miR-192 and miR-21 were the most highly expressed of the active UC–associated miRNAs in human colonic tissues.

To identify miRNA targets in UC patients, genome-wide mRNA microarray analyses were performed. Overall, 876 genes were increased in active UC patients and 267 genes were decreased. Because miR-192 is expressed in the colonic epithelium in active UC and was decreased in these tissues, focus was placed on identifying genes with increased expression in the colonic epithelium. Among the increased genes were 12 colonic epithelial–derived cytokines and chemokines. Of these, macrophage inflammatory peptide-2α (MIP-2α), a chemotactic cytokine, was found to have a putative binding site for miR-192, and immunohistochemistry confirmed its expression in the colonic epithelium and scattered lamina propria mononuclear cells in active UC tissues. Utilizing a human colon cancer–derived cell line (HT29), luciferase reporter assays confirmed binding of miR-192 to this chemokine's 3'UTR. Stimulation with TNF-α resulted in increased MIP-2α expression and decreased miR-192 expression. Experiments with an miR-192 mimic demonstrated that this miRNA could block the TNF-α–induced stimulation of MIP-2α expression.

Furthermore, miRNA microarray analysis and reverse transcription polymerase chain reaction (RT-PCR) validation of the active UC patient group compared to the inactive UC group revealed distinct miRNA expression patterns in patients with active versus inactive UC. Of the 3 miRNAs that were decreased in active UC, miR-192 was unchanged in inactive UC compared to healthy patients, while miR-275 and miR-422b were increased in

inactive UC. Of the 8 miRNAs with increased expression in active UC, 4 had similar expression levels in inactive UC (miR-23a, miR-16, miR-24, and miR-29a) while the expression of miR-21, miR-126, miR-195, and let-7f was more consistent with the levels seen in control individuals. This difference suggests that some miRNAs may be involved in the chronic, dysregulated immune response, while other miRNAs are involved only in acute inflammation.

Given that UC and CD differ in their clinical presentations, genetic associations, gene expression patterns, and immune responses, differing miRNA profiles are expected for these 2 conditions. A study by our laboratory investigated miRNA expression in CD patients.[62] miRNA microarray analysis followed by RT-PCR confirmation was performed on sigmoid colon pinch biopsies from 5 patients with chronically active CD and 13 control individuals. This comparison revealed that expression of miR-23b, miR-106, and miR-191 was increased in tissues from patients with active CD, while miR-19b and miR-629 were decreased in Crohn's colitis patients. An assessment of miRNA expression in terminal ileum biopsies from 6 patients with chronically active terminal ileal CD and 6 control individuals revealed that miR-16, miR-21, miR-223, and miR-594 were overexpressed in chronically active terminal ileal CD tissues.

None of the Crohn's colitis–associated miRNAs were altered in UC tissues. Of the 4 miRNAs altered in Crohn's ileitis, 2 (miR-21 and miR-16) were also altered in UC.[61,62] In addition to its increased expression in Crohn's ileitis and UC sigmoid tissues, miR-21 is increased in other inflammatory disorders such as psoriasis and atopic eczema, and in PBMCs from SLE and ITP patients.[51,55] miR-16 is increased in CD patients, UC patients, and in peripheral blood from RA patients.[63] miR-106 expression was also found to be increased in psoriasis and Crohn's colitis, while miR-223 was increased in Crohn's ileitis and in T cells from RA patients.[51,64] Given these common changes in miRNA expression among immune-mediated disorders, these miRNAs may be involved in targeting central components of the innate and adaptive immune system.

Distinct MicroRNA Profiles in Peripheral Blood

While differential miRNA expression in the terminal ileum and colon may yield important insights into the mechanisms of dysregulated immune activity and inflammation in IBD, the need to collect colonic biopsies for miRNA expression profile analysis makes this an invasive method for diagnosis. In contrast, circulating miRNAs provide easily obtainable specimens that hold greater promise for diagnostic use. Recently, detection of miRNAs in the blood has been extended to immune disorders.[55,63,65] Given that the mechanisms of these diseases include the white blood cell lines, these studies have been performed on whole peripheral blood or PBMCs rather than plasma alone. Recently, unique miRNA expression profiles in PBMCs have been described in RA and SLE.[55,56,63,65]

A study from our laboratory demonstrated that CD and UC differ not only in their tissue miRNA profiles but also in their peripheral blood miRNA profiles.[66] In this study, miRNA microarray analysis was performed on patients with endoscopically and histologically confirmed active CD, inactive CD, active UC, and inactive UC. Four miRNAs (miR-199a-5p, miR-362-3p, miR-532-3p, and miRplus-E1271) were increased and 1 miRNA (miRplus-F1065) was decreased in the peripheral blood of patients with active CD but not in the blood of patients with inactive CD, compared to healthy controls. Both patients with active CD and patients with inactive CD had increased expression of miR-340* and decreased expression of miR-149*.

In UC patients, 9 miRNAs (miR-28-5p, miR-151-5p, miR-199a-5p, miR-340, miRplus-E1271, miR-3180-3p, miRplus-E1035, miRplus-E1153, and miRplus-F1159) were increased in the peripheral blood of patients with active UC but not those with inactive UC. miR-103-2*, miR-262-3p, and miR-532-3p were increased in the blood of both inactive and active UC patients. miR-505* was decreased in the blood of both active and inactive UC patients. Eleven miRNAs (miR-28-5p, miR-103-2*, miR-149*, miR-151-5p, miR-340*, miR-505*, miR-532-3p, miRplus-E1153, miR-3180-3p, miRplus-E1035, and miRplus-F1159) could distinguish active CD from active UC. Of these miRNAs, 10 were significantly increased in active UC compared to active CD, while miR-505* was significantly decreased in active UC. In addition to distinguishing active UC from active CD, the levels of 4 miRNAs (miRplus-E1153, miR-3180-3p, miRplus-E1035, and miRplus-F1159) could distinguish patients with active UC from healthy controls.

Among the 14 patients in the active CD group, miRNA expression did not differ significantly between the Crohn's ileitis and Crohn's colitis subgroups. Similarly, when the 13 patients in the active UC group were subdivided into pancolitis and distal colitis phenotypes, no significant differences were observed between the miRNA expression patterns in these 2 subgroups. Thus, differences in peripheral blood miRNA expression did not appear to be related to the distribution of disease activity.

The expression levels of miR-199a-5p, miR-362-3p, miR-340*, miR-532-3p, and miRplus-1271 were elevated in the blood of both CD and UC patients compared to controls. The fact that expression of these miRNAs is increased in both diseases suggests that these miRNAs may be involved in the regulation of a generalized inflammatory state common to other inflammatory

disorders. Indeed, miR-199a-5p was previously found to be elevated in the PBMCs of African American patients with SLE.[55]

Reports by Glinsky suggest that SNPs associated with bipolar disease, coronary artery disease, CD, RA, type I diabetes, and type II diabetes have homology to miRNAs.[67] Glinsky hypothesized that the DNA sequence alterations associated with many human disorders may contribute to disease phenotypes by altering the function and/or biogenesis of miRNAs.[67,68] Specifically, 6 miRNAs (miR-558, miR-125, miR-199, miR-519, miR-147, and miR-181) have homology to CD-related SNPs, and each of these miRNAs is also homologous to SNPs associated with at least 2 of the diseases mentioned above. Glinsky also reported that miRNAs homologous to disease-linked SNPs are associated with the *NLRP1* and *STAT4* genes.[68] Both miR-337 and miR-588 have high predicted targeting potency to NLRP1, and microarray analysis confirmed that NLRP1 mRNA is decreased in the PBMCs of patients with CD and RA. In contrast, miR-186 and miR-59 have lower predicted mRNA targeting potency, and microarray analysis revealed increased NLRP3 mRNA expression in the PBMCs of CD and RA patients.

Of the 11 miRNAs associated with active UC, the 5 miRNAs associated with Crohn's colitis, and the 4 miRNAs associated with Crohn's ileitis tissue biopsies, none were found to be differentially expressed in the peripheral blood of IBD patients in our study.[61,62,66] Since miRNAs in peripheral blood likely reflect expression in circulating white blood cells, this miRNA expression would not be expected to be the same as that seen in ileal and colonic epithelial cells. On the other hand, differential whole blood miRNA expression is expected between CD and UC, since these 2 diseases differ in their associated T- and B-cell subtypes. Finally, since the whole blood miRNAs found to be associated with UC and CD are not the same as those found in other inflammatory disorders, these miRNAs likely contribute to the unique pathogenic mechanisms of these diseases rather than being part of a generalized inflammatory response.

MicroRNAs in the Development of Inflammation-associated Dysplasia

Long-standing IBD leads to increased colorectal cancer susceptibility in UC and CD patients.[69,70] miRNAs are known to be involved in oncogenesis via their regulation of tumor suppressors and oncogenes.[36] With the recently described role of miRNAs in inflammation and the immune response, the question arises whether changes in miRNA expression may mediate the progression from inflamed tissue to dysplasia and carcinoma.

In a study by Olaru and colleagues, miRNA microarray analyses of 8 chronically inflamed and 8 IBD-associated dysplastic rectal tissues identified 32 miRNAs that were increased and 10 that were decreased in IBD dysplasia.[71] Of the miRNAs that were increased, miR-31 had increased expression in both unaffected and inflamed colonic tissue from IBD patients compared to controls, and miR-31 expression was higher in the affected IBD tissue than the unaffected tissue. No difference in miR-31 expression was found between the IBD dysplasia and IBD carcinoma groups. Thus, miR-31 expression seems to change in a stepwise fashion as tissue goes from normal to chronically inflamed to actively inflamed to neoplastic.

miR-31 expression was also significantly increased in sporadic colorectal cancer specimens compared to normal specimens, although miR-31 expression was lower in sporadic colon cancers than in IBD-associated neoplasia. miR-31 expression levels were able to differentiate IBD-associated neoplasia from normal colonic, unaffected tissue from IBD patients and from inflamed tissue from IBD patients.

To understand the function of miR-31, a computer analysis was performed which identified a hydroxylase called factor-inhibiting hypoxia inducible factor 1 (FIH-1) as a putative target of miR-31. A construct containing the FIH-1 3'UTR and a luciferase reporter confirmed binding of miR-31 to FIH-1. In addition, transfection of human colon cancer–derived HCT116 cells with an miR-31 mimic decreased FIH-1 protein levels. Given that tumor angiogenesis is mediated by hypoxia through activation of the hypoxia inducible factor (HIF) pathway and that FIH-1 catalyzes the post-translational modification of HIF in such a way as to cause its degradation and limit its activity, a decrease in FIH-1 suggests a possible mechanism through which miR-31 could affect tumor angiogenesis.

Dysplasia in IBD patients can be difficult to identify, as identification has traditionally occurred via surveillance colonoscopy with random biopsies. However, newer methods are now utilizing dye spraying, digital filtering, and the addition of magnification or confocal microscopy to the colonoscope to improve the endoscopist's detection of key areas to biopsy.[72] In addition, miRNA expression that increases as tissues progress from inflamed to dysplastic may provide an objective measurement of the progression of this condition and aid in the detection of dysplasia.

Conclusion

IBD is the result of complex interactions between host genetic susceptibility, environmental factors, and immune dysregulation. Post-translational modification of gene

expression by miRNA represents a part of this interaction. miRNA is increasingly recognized as an important element in the development of the innate and adaptive immune system, and changes in miRNA expression are described in many immune-related diseases. Unique miRNA expression profiles exist in active UC, Crohn's ileitis, and Crohn's colitis. The peripheral blood of UC and CD patients also shows differences in miRNA expression profiles. In addition, miRNA expression changes in a stepwise fashion as tissue progresses from being normal, noninflamed tissue in an individual with IBD, to inflamed tissue in an IBD patient, to dysplastic tissue.

The role of miRNA in IBD represents a new pathway for discovery of disease mechanisms, diagnostics, and therapeutics. While changes in miRNA expression and gene expression in IBD have been identified, many of the mechanistic links between miRNA alterations and gene targeting remain to be determined. While UC and CD represent distinct diseases with some overlap, identification of distinct miRNA expression profiles may provide an early method to determine a patient's disease course. After the functional consequences of alterations in miRNA expression are established, miRNA may also become the target of future treatments.

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